VOL. XIV, No. 5 SEPTEMBER-OCTOBER, 1953

610.5
B73

Medical

NOV 20 1953

BORDEN'S REVIEW

of

NUTRITION RESEARCH

AMINO ACID REQUIREMENTS OF MAN

BORDEN'S REVIEW
of
NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published bi-monthly by THE BORDEN COMPANY; officers of the Company: Theodore G. Montague, president; E. L. Noetzel, treasurer; D. T. Orton, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

AMINO ACID REQUIREMENTS OF MAN

By JAMES B. ALLISON, Ph.D.*

Proteins are synthesized in the living system from twenty or more different building blocks, organic compounds of nitrogen called amino acids. This process of synthesis, termed anabolism, results when amino acids are joined one to another through a linkage designated as the peptide bond. Thus long chains of amino acids are formed, chains which may become associated through cross linkages of various kinds. Some of these combined chains form the insoluble proteins of fibrous tissues, of silk, of hair; some are dynamic as the muscle proteins, contracting and relaxing with the shortening and lengthening of the muscle fiber; others are globular, the chains winding into characteristic patterns of the proteins of plasma and cytoplasm. Still others become associated with nucleic acid to perpetuate the genetic characteristics of the animal. Nearly all protoplasmic proteins take part in the enzyme systems of the body, systems which include inorganic ions, vitamins, and other organic structures. Proteins appear in additional specialized roles such as oxygen carrying hemocyanin of the blue-blooded invertebrates, as oxygen carrying hemoglobin of the red-blooded animals, as hormones, as antibodies to disease. Indeed all of the activities of the living system may be correlated with the presence of protein.

Anabolism vs. Catabolism

The complex process of synthesis of proteins from amino acids (anabolism) cannot be set apart from the reverse process of protein breakdown (catabolism). A continuous turnover of protein takes place in the body, an expression of integrated anabolic and catabolic processes, one process being dependent upon the other. Whipple, Robscheit-Robbins, Madden, Miller and co-workers (1) have expressed this dynamic state as an "ebb and flow" between the proteins of the various tissues of the body, a concept of movement which has been demonstrated dramatically by Schoenheimer, Rittenberg, Shemin and associates (2, 3). By using the heavy isotope of nitrogen they followed the turnover of nitrogen from one tissue protein to another, an experimental feat which has been repeated with other isotopes of atoms found in amino acids such as deuterium, radioactive carbon C^{14} and sulfur S^{35}. Thus it can be said that the proteins of the liver, blood, kidneys, heart,

*Bureau of Biological Research and Department of Physiology and Biochemistry, Rutgers University, New Brunswick, N. J.

muscles and other soft tissues of the body are interchanging, tissue proteins being continually broken down and reconstructed, but the rates of turnover of the various proteins differ markedly.

Some scientists like to picture the body as containing an integrated metabolic pool of amino acids, contributed to by the diet, by synthesis, by tissue protein breakdown. From the pool could come all of the amino acids or derivatives needed for anabolic and catabolic processes. By making this assumption, and by using data obtained from isotope studies, a metabolic pool for man has been estimated to be equivalent to 0.5 gram of amino acid nitrogen per kilogram of body weight and the rate of synthesis of protein to involve 0.2 gram of amino acid nitrogen per kilogram per day (3).

The "half-life" of tissue proteins may be calculated also from the turnover of isotopic atoms. The average half-life of body proteins in man has been estimated to be 85 days. Some tissue proteins, however, are broken down and regenerated more rapidly than this, *eg.* liver and serum proteins, while muscle protein on the other hand has a half-life close to 180 days (3).

This dynamic state does not represent simply a shift of amino acids from one protein to another or from one tissue to another without loss. The process of tissue breakdown and regeneration results in the irreversible destruction of some of the amino acids, an essential destruction which yields energy to the living system. Urinary urea nitrogen is the end waste product of this catabolism of amino acids. The amino acids destroyed must be replaced by the diet or be synthesized in the body from constituents in the diet. If amino acids are lacking in the food, catabolism results in the reduction of the amino acids supply of the body, thereby in a decrease of tissue proteins. The proteins with the greatest turnover, such as those of the liver, intestinal mucosa, and the blood are depleted most rapidly, the greatest storehouse being represented by the soft tissues of the body (4).

The greater the magnitude of those stores, the greater the supply of tissue amino acids which can be called upon for energy and for maintenance of essential structures, a supply that must be drawn upon during periods of starvation or of illness, or of dietary errors of many kinds. Thus catabolism of tissue amino acids to form the waste product urea is continuous, the amount being high when the protein stores and metabolic activity are high, the amount being low when the stores and activity are low. Evidence is accumulating to indicate that high protein stores with this high catabolic activity give energy and reserves to meet the stresses and strains of living.

Urinary creatinine is also an end product of tissue catabolism. Reactions involving the amino acids glycine, arginine, and methionine lead to the formation of creatine which is dehydrated to form creatinine during processes of energy transfer in the muscle. In general, the twenty-four hour ex-

cretion of creatinine is constant and a function of the muscle mass of the individual, and is independent of the diet unless it contains unusually large amounts of the precursors for creatine. Folin's idea of endogenous tissue protein catabolism (5, 6) was based on the independence of the excretion of creatinine to diet, endogenous referring to tissue nitrogen, exogenous to dietary nitrogen.

The excretion of creatinine, however, is not only independent of the diet but also of the utilization of labile tissue protein stores; the excretion being approximately the same when the stores are depleted as when they are maximal (7). Possibly creatinine formation is correlated with the number of functional nuclei in the muscles, a number which is essentially fixed in the embryo and cannot be altered by diet. Regeneration of depleted muscle tissues in the adult means adding protein to the cells already potentially functional and in existence, but not in generating new cells.

Essential Amino Acids

Some living systems, like the plants, can manufacture all of the amino acids needed for anabolism from dietary inorganic nitrogen and carbon. Others, like certain bacteria, can manufacture all amino acids from a single amino acid. Still others, like man, can manufacture only a portion of the amino acids from dietary sources to meet the needs for maintenance and growth. Those amino acids which are not synthesized in adequate amounts and must therefore be supplied *de novo* in the diet have been called essential by Rose (8). He has demonstrated that normal adult man requires eight amino acids in the diet to meet the demands for replacement of tissue catabolism, that is for maintenance of the status quo of protein stores. These essential amino acids, together with tentative recommendations for daily intakes, are recorded in Table I (8). The intakes are double those found to be minimum requirements, doubling being recommended by Rose to insure an ample supply.

The recommended intakes, it will be noted, are based on the assumption that the diet furnishes sufficient nitrogen for the synthesis of non-essential amino acids. Rose and co-workers (8, 9), Frost (10), and others have reported that the addition of essential amino acids as the sole source of dietary nitrogen cannot support the growth of rats as well as a diet containing protein or protein products. Albanese (11) also emphasizes the physiological value of feeding a mixture of the essential amino acids with the non-essentials.

The patterns needed for maintenance of the status quo of protein stores in the adult may differ from the pattern needed for growth and for wound

healing (12), for lactation, for pregnancy (13), for convalescence. The dividing line between essential and non-essential is not always sharp and the variety and amount of amino acids needed by an individual may vary with the physiological state. Ten amino acids are essential, for example, to promote adequate growth of the young rat, eight of them being the same as those in Table I. Arginine and histidine are the other two (14). Frazier *et al* (15) and Benditt *et al* (16) found that, with the exception of arginine, these same acids were essential for repletion of the protein stores of the depleted adult rat. Burroughs, Burroughs and Mitchell (17), on the other hand, concluded that only 5 of the 10 amino acids are essential for maintenance of the protein stores in the adult rat, these acids being tryptophan, threonine, isoleucine, valine and methionine. More recently Mitchell (18) and Henry and Kon (19) have re-emphasized the fact that lysine is not as essential to the adult as it is to the growing rat. Wissler *et al* (20), however, have presented data to show that the adult rat requires all of the essentials save arginine, if appetite, weight, and overall "adult growth" are to be promoted. Similarly Kliger and Krehl (21) found that small amounts of lysine were required for the maintenance of body weight in adult rats. An appreciation of the difficulties in determining the essential amino acids needed for maintenance of protein stores may be gained by a review of the significance of nitrogen balance, the measurement most often used to determine maintenance.

TABLE I.

Tentative Recommended Intakes of Essential Amino Acids for Normal Adult Man when Diet Furnishes Sufficient Nitrogen for Synthesis of Non-essentials. (Data taken from Rose (8).

Amino Acid	Daily Intake gm.
L-Tryptophan	0.5
L-Phenylalanine	2.2
L-Lysine	1.6
L-Threonine	1.0
L-Valine	1.6
L-Methionine	2.2
L-Leucine	2.2
L-Isoleucine	1.4

The Concept of Nitrogen Balance

Nitrogen balance is the difference between nitrogen intake and nitrogen excreted. Nitrogen balance may be defined mathematically as follows:

$$NB = NI - (UN + FN)$$

where NB = nitrogen balance, NI = nitrogen intake, UN = urinary nitrogen and FN = feces nitrogen (22). If nitrogen intake is greater than the nitrogen excreted then the body is gaining in nitrogen and is in positive balance. If the nitrogen intake is just equal to nitrogen excreted then the body is maintaining nitrogen and is said to be in equilibrium. If nitrogen intake is less than nitrogen excreted then the body is losing nitrogen and is in negative balance.

The amount of dietary nitrogen needed to maintain nitrogen equilibrium will vary with the magnitude of the protein stores and with the ability of the dietary protein to furnish the amino acids in amounts and kinds needed for anabolism, factors in maintenance which were emphasized by Melnick and Cowgill several years ago (23). Thus it is possible to establish equilibrium at various levels of nitrogen intake.

The high excretion of nitrogen from the catabolism of protein stores of the normal dog fed a protein-free diet is illustrated in Table II (24), a

TABLE II.

Data obtained while feeding dogs a diet containing various protein sources, the dogs being first in a state of normal protein stores followed by a state of depleted stores (24).

Dietary Protein	Urinary nitrogen protein-free diet	Urinary nitrogen at equilibrium	Dietary nitrogen to produce nitrogen equilibrium
Normal Protein Stores			
Egg White	2.0	1.8	2.1
Casein	1.9	2.7	3.1
Wheat Gluten	2.2	5.8	6.2
Depleted Protein Stores			
Egg White	1.2	1.1	1.3
Casein	1.1	1.4	1.7
Wheat Gluten	1.2	1.9	2.3

relatively large amount of nitrogen being required to maintain equilibrium in these animals. The excretion of nitrogen in the depleted dog was low and it required little nitrogen to maintain equilibrium. The excretion of urinary nitrogen while feeding the egg white to the normal dog was not increased above the excretion on a protein-free diet. Such data can be interpreted to mean that all of the amino acids presented by the egg white were utilized for regenerating tissue protein lost through daily tissue catabolism. More casein nitrogen than egg white nitrogen was needed to maintain equilibrium and the excretion of urinary nitrogen was increased above that recorded for a period of protein-free feeding. Thus it can be said that a portion of the pattern of amino acids supplied by casein was not utilized for tissue regeneration but was catabolized, thereby increasing the excretion of urea nitrogen in the urine. Still more wheat gluten nitrogen was needed for nitrogen equilibrium. The excretion of urinary nitrogen was more than double that observed during a period of protein-free feeding, a still larger portion of the pattern of amino acids presented by wheat gluten being catabolized and not used for synthesis. It will be noticed, however, that the differences between the amounts of the various protein sources needed for nitrogen equilibrium was less in the depleted than in the normal animal. Reference will be made later to data which support the concept that potential anabolic activity increases as the individual is depleted in protein stores and that different patterns of amino acids may do different jobs of repletion, some promoting the filling of one store more than another.

Maintenance of nitrogen equilibrium, therefore, does not necessarily mean that adequate dietary protein is being fed. It simply means that the individual is being maintained for a time in nitrogen equilibrium, possibly even maintained in a depleted state of imbalance in protein stores. The body adapts to nitrogen intake so that if the intake is too low to maintain equilibrium the individual will go into negative balance, protein stores will be depleted until the small amount of dietary nitrogen will maintain equilibrium in a depleted state. Similarly if the intake is high, positive balance will be produced, the stores will be filled to the point where the high dietary intake will become essential to maintain those stores. The larger the protein stores, the higher the metabolic activity, the more dietary nitrogen is needed to maintain nitrogen equilibrium.

It should be emphasized that nitrogen balance is the sum of gains and losses of nitrogen from all the tissues of the body so that it is possible for an individual to be in positive balance, that is to be gaining in nitrogen but still to be losing nitrogen from some tissues. Such a shift in nitrogen from one tissue to another can be illustrated in an exaggerated form in an indi-

vidual with cancer. Positive balance can be produced but some tissues of the body will be losing while the cancer tissue is gaining nitrogen (25). The problem of amino acid nutrition at the cellular level has been emphasized by Christensen (26) who discusses the factors involved in providing every cell with adequate levels of each acid, the main factor being "the activity of the cells in taking up amino acids, apparently against concentration gradients."

Effect of Food Processing and Digestion

Although animals can be maintained by feeding the essential amino acids by vein (4, 27) or by mouth, the normal presentation of amino acid mixture to the body is through digestion (See review of Protein Nutrition by Flodin, 28). Cooking and rates of digestion may improve or reduce the nutritive value of proteins according to the effect these processes have upon the liberation of amino acids for absorption into the body. Cannon, Geiger (29, 30) and others have emphasized the importance of absorption of all of the essential amino acids simultaneously and in proper proportions from the intestinal tract.

Numerous reports have appeared demonstrating that heating of soy products and other legumes increases their nutritive value, whereas excessive heating reduces the nutritive values of many proteins. Block *et al* (31), for example, prepared a cake that supplied about the same distribution of essential amino acids as found in eggs. If this cake was baked dry or toasted the nutritive value was reduced. Addition of the essential amino acid lysine to the toasted cake restored the nutritive value. The authors suggested that lysine was not liberated properly from the toasted cake in the gastrointestinal tract. Melnick and Oser (32) have reviewed the extensive literature on the effect of food processing on nutritive value, and they present data to demonstrate that heating alters enzymatic digestion so that methionine may be liberated more rapidly and in better proportions with the other amino acids from soy products whereas heating may reduce the rate of liberation of lysine.

The importance of simultaneous availability of essential amino acids is illustrated also by feeding mixtures of proteins, one protein supplementing the amino acid deficiency in the other. Such proteins fed simultaneously result in better growth of rats than when they are supplied at separate feedings (33-35). Then too a proper distribution of feedings is very important, division of the complete dietary protein mixture into the several meals sometimes being more efficient than presentation of the same amount of proteins at two meals or less (36-38).

Amino Acids from Food Proteins

Nitrogen balance is, therefore, a function of the utilization of dietary amino acids, the dietary proteins simply acting as a vehicle to carry the acids into the gastrointestinal tract where they must be liberated by digestion for proper absorption into the body. The data in Table III illustrate the amino acid patterns of six protein sources selected for a cooperative study by a group of laboratories throughout the United States. The amino acid values given in this table are the "best" estimates obtained from data provided by twelve cooperating laboratories (39). The data in Table IV illustrate amino acid patterns in other common food stuffs, data taken from Block and Bolling (40).

TABLE III.

Amino acid pattern of six protein sources selected for cooperative study (39).
gm. of amino acid/16 gm. nitrogen (100 gm. protein)

Amino Acid	Beef*	Labco casein	Egg albumin	Egg** powder	Peanut flour	Wheat gluten
Arginine	6.4	3.9	6.0	6.2	11.3	3.7
Histidine	3.2	3.0	2.4	2.1	2.2	2.0
Lysine	8.6	8.1	7.2	6.1	3.5	2.0
Tryptophan	1.0	1.0	1.1	1.1	0.8	0.8
Phenylalinine	3.9	5.4	5.9	5.6	4.9	5.0
Cystine	1.3	0.4	3.0	2.3	1.4	2.4
Methionine	2.7	3.2	4.2	3.2	0.8	1.7
Threonine	4.4	4.5	4.9	4.9	2.8	2.8
Leucine	7.8	10.0	8.8	9.0	7.1	7.2
Isoleucine	5.2	6.3	6.4	6.2	4.1	4.5
Valine	5.1	7.4	7.5	7.0	4.6	4.3
Aspartic Acid	9.3	7.3	11.0	10.8	13.0	3.8
Glutamic Acid	14.6	22.4	12.9	12.6	18.3	35.0
Glycine	5.6	2.0	3.8	4.0	5.4	3.3
Tyrosine	3.0	5.2	3.3	3.5	2.9	2.8
Proline	5.1	13.4	4.3	4.5	5.0	14.1

*dehydrated, defatted beef representing the muscle meat of the whole animal.
**dehydrated, defatted whole egg powder.

TABLE IV.

Amino acid composition of some food proteins
gms. of amino acid 16.0 gms. nitrogen (100 gm. protein)
Data taken from Block and Bolling, 2nd ed. (40)

Amino acid	Whole egg	Whole milk	Lact-albumin	Cheese	Soy-bean	Muscle animal	Muscle fish	Liver	Bread	Wheat flour	Whole wheat	Whole corn	Gel-atin
Arginine	6.6	4.2	4.0	3.6	7.3	7.7	7.4	6.6	3.5	3.9	4.3	4.8	8.2
Histidine	2.4	2.6	2.3	3.3	2.9	3.3	2.6	2.5	2.3	2.2	2.1	2.5	0.9
Lysine	7.0	8.7	10.5	8.5	6.8	10.0	9.0	7.0	2.8	1.9	2.7	2.3	5.0
Tryptophan	1.5	1.5	2.5	1.6	1.4	1.4	1.2	1.5	1.3	0.8	1.2	0.6	0.0
Phenylalanine	6.3	5.5	5.0	6.4	5.3	5.0	4.4	6.1	5.0	5.5	5.1	5.0	2.3
Cystine	2.4	1.0	4.0	0.4	1.9	1.2	1.2	1.4	2.1	1.9	1.8	1.5	0.1
Methionine	4.0	3.2	2.6	3.5	1.7	3.2	3.2	3.2	2.0	2.0	2.5	3.1	0.8
Threonine	4.3	4.7	6.0	3.7	3.9	5.0	4.7	5.3	2.8	2.7	3.3	3.7	1.9
Leucine	9.2	11.0	12.1	9.0	8.0	8.0	9.5	8.4	—	7.0	7.0	15.0	3.5
Isoleucine	7.7	7.5	7.5	7.3	6.0	6.0	6.5	4.8	—	4.2	4.0	6.4	1.7
Valine	7.2	7.0	6.6	7.7	5.3	5.5	6.0	6.0	—	4.1	4.3	5.3	2.8
Aspartic Acid	—	—	11.0	—	—	7.8	—	6.9	—	—	—	—	6.2
Glutamic Acid	—	21.5	18.0	—	18.4	15.4	—	10.6	—	—	29.0	—	11.0
Glycine	2.7	2.3	3.5	—	—	5.0	—	8.0	—	7.0	—	—	23.6
Tyrosine	4.5	6.0	5.3	6.8	4.0	4.0	3.8	3.9	4.4	3.8	4.0	6.0	0.5

Nutritive Value of Proteins (28)

Although the determination of the nutritive value of dietary proteins may be complicated by food preparation and methods of feeding, it is ordinarily assumed that the amino acid patterns illustrated in Tables III and IV represent approximately the patterns presented to the body.

The nutritive values of the protein sources recorded in Table III have been determined during growth and maintenance, some of the results being summarized in Table V. These data illustrate again the difference between the patterns needed for growth and for maintenance in the rat. In row 1, under "for growth," wheat gluten, which is markedly deficient in lysine, has only a value of 8 relative to 100 for egg white, data which were calculated from experiments where gain in weight was the measurement of nutritive value. In row 2, wheat gluten has a higher nutritive value relative to egg white, data which were calculated from the fraction of dietary nitrogen retained in the body of the animal, a fraction which integrates more accurately the requirements for both maintenance and growth. That wheat gluten has a relatively high nutritive value for maintenance is illustrated in the data for adult rats in Table V.

The nutritive value of casein is improved markedly for the dog and for the rat by supplementing the casein with methionine (44, 45). Similarly, by adding lysine, the nutritive value of wheat gluten for maintenance in the dog is increased (24). Thus supplementation of amino acid patterns by adding the deficient amino acid can be used to improve nutritive values (46). Mitchell and Beadles (41) conclude from data such as these and in Table V that the "cystine-methionine requirement of the adult rat is more intense in relation to requirements for other amino acids, than that of the growing rat or of the adult human. On the other hand, the lysine requirement is much less. These differences may be explained on the basis of the amino acid composition of hair and the relative importance of hair growth in the protein nutrition of the growing rat, the adult rat and the adult human." "Except for differences in the proportional requirements for cystine-methionine and lysine, the dietary nitrogen requirement for nitrogen equilibrium is of the same order of magnitude for adult rats and adult humans when expressed as milligrams of nitrogen per calories of basal metabolism."

Whole egg proteins have usually been considered as representing the best natural source for a pattern of amino acids to promote growth as well as maintenance. The lower values for whole egg than for egg white recorded in Table V for rats and dogs may be due to the low cystine-methionine ratio in this whole egg preparation, a deficiency which probably would not be detected in man (47, 48). Other preparations of whole egg tested in rats

TABLE V.

Nutritive value of six selected proteins, the value being expressed relative to egg white as 100 (39).

Animal	Egg white	Whole egg	Beef	Casein	Peanut flour	Wheat gluten
For Growth						
Rat	100	95	84	80	31	8 (39)
	100	90	78*	71*	56	41 (41)
For Maintenance						
Rat	100	87	73	54	49	69 (41)
Dog	100	76	68	64	49	39
Man	100	103	74	74	62	46 (42)

*Taken from Block and Mitchell (43).

and dogs have had cystine-methionine ratios and nutritive values nearer to egg white. The superiority of protein sources such as eggs, milk, and meat, illustrated in Table V for man have been well established by the extensive work of Murlin, Nasset and associates (49).

Mutual supplementation of proteins through combination of foods is a common practice. Mixtures of lactalbumin and casein or whole milk proteins do not have the cystine-methionine deficiency observed in casein when fed to rats and to dogs. Cereal proteins with a deficiency in lysine may be supplemented with proteins of animal sources. It is interesting to compare the amino acid patterns which have been proposed for maintenance in the adult with the patterns consumed by the different populations the world over. The numbers in the first column in Table VI were calculated from the data for maintenance proposed by Stare, Hegsted, and McKibbin (50), an estimate being made that 25 grams of a dietary protein containing this pattern would maintain nitrogen equilibrium in an average 70 kg. man, under usual short term experimental conditions. Similarly the data of Harte and Travers (51) and of Rose (8) for maintenance have been converted into a pattern to be supplied by 25 grams of dietary protein, this form of presentation being useful for comparison with actual patterns consumed by people and illustrated in the remainder of Table VI. It should be emphasized, however, that Rose (8) and Mitchell (52) have recommended approximately double this intake for maintenance to assure an ample supply and to take care of "adult growth." It is customary to assume that 1 gram of ordinary mixed dietary

TABLE VI.

Pattern of Amino Acids for maintenance calculated as gm/100 gm of protein			
Amino acid	Stare et al (48)	Harte and Travers (49)	Rose (8)
Arginine	7.2	4.8	—
Histidine	2.0	2.0	—
Lysine	5.6	3.2	3.2
Tryptophan	1.6	1.6	1.0
Phenylalanine	5.6	5.6	4.4
Cystine and) Methionine)	7.6	3.0	4.4*
Threonine	4.8	4.0	2.0
Leucine	14.4	6.8	4.4
Isoleucine	5.2	4.8	2.8
Valine	5.2	4.4	3.2

*methionine only.

Patterns of Amino Acids consumed by humans gm/100 gm protein.

U.S. Army (Block)	Middle class rural U.S. (Block)	urban U.S. (Block)	Japanese rural (Block)	Indian diet (Block)	Average data from Block and Autret (55) (Block)
6.6	5.7	5.7	—	7.2	5.5
2.4	2.3	2.3	—	1.7	2.3
7.0	5.8	5.4	5.2	3.2	4.2
1.5	1.3	1.2	1.2	1.3	1.2
6.3	5.5	5.5	—	5.0	4.8
6.4	4.1	4.1	3.6	4.3	1.8*
4.3	3.9	3.8	3.9	3.8	3.8
9.2	9.0	8.8	7.5	8.2	8.9
7.7	6.0	5.7	5.9	5.2	4.6
7.2	5.9	5.7	4.9	6.2	5.5

protein per kg. of body weight should be included in the diet of man, but even this estimate could be on the low side if the quality of protein is low or if the need for growth or regeneration of tissues (53-56) is high. Dogs, for example, may be maintained in nitrogen equilibrium and apparently in good condition when receiving 1 gm. of casein per kg. of body weight. Their protein stores are increased and they are more resistant physiologically, however, if fed double this amount.

The data in Table VI indicate that if the intake was sufficiently high the patterns of amino acids consumed the world over would be adequate to supply the essential amino acids recommended for maintenance of nitrogen balance (8). The last column records a pattern calculated from data presented by Brock and Autret (56) in relation to their study of Kwashiorkor. Such averages as these, however, may be misleading because it is the diet of the individual that counts. Acknowledgment is made to Dr. R. J. Block for some of the values recorded in Table VI. He has pointed out (personal communication) that the low value for methionine, calculated from the data of Brock and Autret (55), does not mean that this acid is a limiting factor in the diet. Cystine, which was not determined, could make up the apparent deficiency in sulfur amino acids since in mixed foods of vegetable origin the cystine content is usually equal to methionine. The limiting acid, as Block has emphasized, a limitation mentioned also by the authors, is more likely to be the low lysine content of the mixed vegetable diet.

The Problem of Excessive Catabolism

Nutritional errors, such as low calorie intake, deficiency in dietary protein, starvation, lack of certain vitamins, or almost any illness will increase loss of tissue nitrogen through excessive catabolism (57, 58). Excessive catabolism causes loss in tissue proteins producing an imbalance in many tissues. In the blood, for example, the total circulating plasma albumin decreases while total circulating α and β globulins may not change or may even increase (59). The γ globulin which contains antibodies to disease also drops markedly (60).

Reduced total protein in the blood is called hypoproteinemia and is one test used to detect depletion in protein stores. Depletion can take place, however, without the development of hypoproteinemia because as the water holding albumin is decreased plasma volume drops. If the drop in plasma volume is sufficiently great the concentration of protein in the blood can remain normal (7). Similarly loss of water may occur in other tissues of the body such as the liver. This shift in water balance, associated with de-

pletion, results in the accumulation of fluid in extra-cellular spaces which, if severe, can be detected clinically as nutritional edema.

Proteins lost from tissues represent loss in enzyme systems. At least 30 enzyme systems have been assayed by various investigators in the tissues of animals depleted in protein stores, the total activity of many enzyme systems being decreased as the stores are decreased. Thus the overall potential metabolic activity is reduced but more than this, a marked imbalance in the activity of enzyme systems is produced in some of the tissues. Depletion in the rat liver, for example, does not reduce the activity of phosphatase but rather increases it and also increases slightly the activity of cytochrome oxidase per unit of liver nitrogen. On the other hand, depletion does reduce the unit activities of other oxidases in the following order: Xanthine oxidase > diphosphopyridine nucleotide cytochrome c reductase > uricase = succinic dehydrogenase > succinic oxidase > d-amino acid oxidase (61, 62).

The liver and gut muscle are ordinarily depleted most rapidly by excess catabolism, but under certain conditions they are not. In the presence, for example, of a growing tumor which is depleting other tissues of the body, the stores of the liver may remain at a high level, possibly contributing thereby to the high anabolic needs of the growing tumor. Indeed tumors have been classified in our laboratories as to their malignancy in terms of depletion of normal tissues. A very malignant tumor will deplete the animal even in the presence of maximum diet and will be little affected by placing the animal on a protein-free diet. A less malignant tumor will be reduced in growth markedly by placing the animal on a protein-free diet (25, 63). In general, it may be concluded that the protein-depleted individual is adapted to a protein deficient diet. Metabolic activity is low, excretion of nitrogen is low, but the energy of an important muscle like the heart is still relatively great (7).

The Problem of Repletion (64, 65)

The protein-depleted individual is, in general, poised for regeneration with the potential anabolic activities greater than in normal individuals (7). Thus a given pattern of amino acids, even one somewhat low in one or more of the essential amino acids is utilized for anabolic purposes much better in the depleted than in the normal individual. The apparent requirement of essential amino acids for maintenance of nitrogen balance may be less in the depleted individual but actually the need is greater. A high dietary protein intake with proper balance of amino acids repletes the body rapidly back to a normal distribution of tissue proteins. The labile protein stores are increased, metabolic activity increases, and much nitrogen is needed to

keep the repleted individual in a condition of equilibrium. An inadequate dietary protein, on the other hand, cannot put the individual as far in positive balance, some protein stores are repleted more rapidly than others, and metabolic activity may even be decreased rather than increased. Dogs, for example, repleted for thirty days by feeding wheat gluten gained more rapidly in plasma proteins than animals repleted in egg proteins, but at the end of the period of repletion the excretion of nitrogen from catabolism of protein stores in the wheat gluten fed dogs was even less than in most depleted animals (24). Chow (66) also found that wheat gluten stimulated the formation of circulating proteins and Albanese (67) has presented data to show that wheat gluten in the diet of infants will promote the formation of plasma proteins, particularly when measured in terms of arginine content.

These data and others suggest that the amino acid pattern derived from different dietary proteins may fill one tissue compartment more rapidly than another. Bovine serum protein, for example, fed in the diet of depleted dogs resulted in the formation of albumin while casein hydrolysate promoted the formation of both albumin and globulin, even increasing above normal the globulin fraction (66, 68). Robscheit-Robbins and Whipple (69) have presented one of the most interesting examples of the effect of dietary proteins upon repletion of blood proteins in dogs. They found that it was possible to control the production of plasma proteins and of hemoglobin by shifting the type of protein in the diet.

An important experiment was done on the growth of puppies, one group being given whole egg protein, the other gluten which was deficient in the amino acid lysine. The puppies fed the gluten actually ate more calories than those fed the whole egg protein diet so that the grams gained in body weight per gram of nitrogen eaten was about the same for both groups of dogs. The dogs fed wheat gluten were fat and not very active whereas those fed egg proteins were lean and very active. Nitrogen balance studies demonstrated that the dogs fed wheat gluten had stored much less nitrogen in their bodies than the others and they were not as well prepared to meet the stresses of living (70).

The Calorie Problem

The body is a machine which demands calories for energy so that if calories are not supplied in adequate amounts in the diet tissue protein will be catabolized. The data indicate that there is an optimum caloric intake for maximum anabolism and that reducing caloric intake below that optimum will cause the individual to dip into tissue proteins; or put in another

way, tissue catabolism will be increased. It is probable that tissue catabolism is increased with even small reductions from so-called normal caloric intakes (71-75). As caloric intake is reduced more and more protein is utilized for catabolism until a point is reached where many nutritionists have felt that none of the dietary protein could be utilized for anabolism. Evidence obtained on animals, however, indicates that even with very low caloric intakes some protein anabolism takes place and the individual does benefit from inclusion of protein in the diet (75, 76).

REFERENCES CITED

1. WHIPPLE, G. H., "Hemoglobin, Plasma Protein and Cell Protein." C. C. Thomas, Springfield, Ill. (1948).

2. SCHOENHEIMER, R., "The Dynamic State of Body Constituents." Harvard University Press, Cambridge (1942).

3. RITTENBERG, D., Harvey Lectures *44,* 200 (1948-49).

4. ELMAN, R., Advances in Protein Chemistry, *3,* 269 (1947).

5. FOLIN, O., *Am. J. Physiol., 13,* 117 (1905).

6. MITCHELL, H. H., "Protein and Amino Acid Requirements of Mammals." Ed. by A. A. Albanese, Academic Press, N. Y. (1950).

7. ALLISON, J. B., *Agri. and Food Chem., 1,* 71 (1953).

8. ROSE, W. C., *Federation Proceedings 8,* 546 (1949).

9. ROSE, W. C., Oesterling, M. J., and Womack, M., *J. Biol. Chem., 176,* 753 (1948).

10. FROST, D. V., "Protein and Amino Acid Requirements of Mammals." Ed. by A. A. Albanese, Academic Press, N. Y. (1950).

11. ALBANESE, A. A., Ibid.

12. WILLIAMSON, M. B. and Fromm, H. J., *Proc. Soc. Exp. Biol. Med., 80,* 623 (1952).

13. SHEFT, B. B., and Oldham, H., *J. Am. Dietetic Assoc., 28,* 313 (1952).

14. ROSE, W. C., *Physiol. Rev., 18,* 109 (1938).

15. FRAZIER, L. E., Wissler, R. W., Steffee, C. H., Woolridge, R. L., and Cannon, P. R., *J. Nutrition, 33,* 65 (1947).

16. BENDITT, E. P., Hamphreys, E. M., Straube, R. L., Wissler, R. W., and Steffee, C. H., *J. Nutrition, 33,* 85 (1947).

17. BURROUGHS, E. W., Burroughs, H. S., and Mitchell, H. H., *J. Nutrition, 19,* 363 (1940).

18. MITCHELL, H. H., *Arch. Biochem., 12,* 293 (1947).

19. HENRY, K. M., and Kon, S. K., *J. Dairy Res., 19,* 216 (1952).

20. WISSLER, R. W., Steffee, C. H., Frazier, L. E., Woolridge, R. L., and Benditt, E. P., *J. Nutrition, 36,* 245 (1948).

21. KLIGER, D., and Krehl, W. A., *J. Nutrition, 46,* 61 (1952).

22. ALLISON, J. B., *Federation Proc. 10,* 676 (1951).

23. MELNICK, D., and Cowgill, G. R., *J. Nutrition, 13,* 401 (1937).

24. ALLISON, J. B., Anderson, J. A., and White, J. I., Trans. Am. Assoc. Cereal Chem. 7, 24 (1949).

25. BABSON, A., "Some Effects of Transplanted Tumors on Protein Interrelations in Rats." Ph.D. thesis, Rutgers Univ. (1953).

26. CHRISTENSEN, H. N., *Ann. Rev. Biochem. 22,* 233 (1953).

27. SILBER, R. H., and Porter, C. C., *J. Nutrition, 38,* 155 (1949).

28. FLODIN, N. W., *Agr. and Food Chem., 1,* 22 (1953).

29. CANNON, P. R., *J. Am. Med. Assoc., 135,* 1043 (1947).

30. GEIGER, E. J., *Nutrition, 34,* 97 (1947).

31. BLOCK, R. J., Cannon, P. R., Wissler, R. W., Steffee, C. H., Jr., Straube, R. L., Frazier, L. E., and Woolridge, R. L., *Arch. Biochem. 10,* 295 (1946).

32. MELNICK, D., and Oser, B. L., *Food Tech., 3,* 57 (1949).

33. HARTE, R. A., Travers, J. J., and Sarich, P., *J. Nutrition, 36,* 287 (1948).

34. GEIGER, E., *J. Nutrition, 36,* 813 (1948).

35. HENRY, K. M., and Kon, S. K., *J. Dairy Res. 14,* 330 (1946).

36. GEIGER, E., El Rawi, I., and Geiger, L. E., *Fed. Proc. 11,* 444 (1952).

37. LEVERTON, R. M., and Gram, M. R., *J. Nutrition, 39,* 57 (1949).

38. MERTZ, E. T., Baxter, E. J., Jackson, L. E., Roderuck, C. E., and Weis, A., *J. Nutrition, 46,* 313 (1952).

39. "Cooperative Determination of the Amino Acid Content, and of the Nutritive Value of Six Selected Protein Food Sources." Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey (1950).

40. BLOCK, R. J., and Bolling, D. "The Amino Acid Composition of Proteins and Foods." Charles C. Thomas, Springfield, Ill., 2nd Ed. (1951).

41. MITCHELL, H. H., and Beadles, J. R., *J. Nutrition, 40,* 25 (1950).

42. HAWLEY, E. E., Murlin, J. R., *J. Nutrition,* 40, 25 (1950).

43. BLOCK, R. J., and Mitchell, H. H., *Nutrition Abs. and Rev. 16,* 249 (1946-47).

44. ALLISON, J. B., Anderson, J. A., and Seeley, R. D., *J. Nutrition, 33,* 361 (1947).

45. RUSSELL, W. C., Taylor, M. W., Mehrhof, T. G. and Hirsch, R. R., *J. Nutrition, 32,* 313 (1946).

46. SURE, B., *J. Nutrition, 50,* 235 (1953).

47. JOHNSON, R. M., Deuel, H. J., Jr., Morehouse, M. G. and Mehl, J. W., *J. Nutrition, 33,* 371 (1947).

48. COX, W. M., Jr., Mueller, A. J., Elman, R., Albanese, A. A., Kemmerer, K. S., Barton, R. W., Holt, L. E., Jr., *J. Nutrition, 33,* 437 (1947).

49. MURLIN, J. R., Szymanski, T. A., and Nasset, E. C., *J. Nutrition, 36,* 171 (1948).

50. STARE, F. J., Hegsted, D. M. and McKibbin, J. M., *Ann. Rev. Biochem. 14,* 431 (1945).

51. HARTE, R. A., and Travers, J. J., *Science 105,* 15 (1947).

52. BRICKER, M., Mitchell, H. H., and Kinsman, G. M., *J. Nutrition 30,* 269 (1945).

53. SHERMAN, H. C., *J. Biol. Chem. 41,* 97 (1920).

54. Food and Nutrition Board, National Research Council Reprint and Circular Series 129 (revised 1948).

55. YOUMANS, J. B., Patton, E. W., Sutton, W. R., Kern, R., and Steinkamp, R., *Am. J. Pub. Health 33,* 955 (1943).

56. BROCK, J. F. and Autret, M. "Kwashiorkor in Africa." World Health Organization Monograph Series 8 (1952).

57. KEYS, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H. L. "Biology of Human Starvation," Vol. I. Minneapolis, University of Minnesota Press (1950).

58. LYNCH, H. D. and Snively, W. D., *J. Am. Med. Assoc. 147,* 115 (1951).

59. CHOW, B. F., *Ann. N. Y. Acad. Sci. 47,* 297 (1946).

60. CANNON, P., Symposia on Nutrition, Vol. II, Plasma Proteins. By the Robert Gould Research Foundation, Inc. Charles C. Thomas, Springfield, Ill. (1950).

61. WAINIO, W. W., Eichel, B., Eichel, H., Person, P., Estes, F. L., and Allison, J. B., *J. Nutrition 49,* 465 (1953).

62. MILLER, L. L., *J. Biol. Chem. 186,* 253 (1950).

63. "Nutrition in Relation to Cancer." Editor, R. W. Minor; Consulting Editor, C. G. King; Assoc. Editor, L. Salin, *Ann. N. Y. Acad. Sci. 49* (1947).

64. POLLACK, H., *Advances in Protein Chemistry 6,* 385 (1951).

65. PETERS, J. P., Elman, R., Co Tui — separate papers in "Proteins and Protein Hydrolysates in Nutrition." *Ann. N. Y. Acad. Sci.* 47 (1946).

66. CHOW, B. F., "Protein and Amino Acid Requirements of Mammals," Academic Press, N. Y. (1950). Ed. by A. A. Albanese.

67. ALBANESE, A. A., *J. Biol. Chem. 200,* 787 (1953).

68. SEELEY, R. D., *Am. J. Physiol. 144,* 369 (1945).

69. ROBSCHEIT-ROBBINS, F. S. and Whipple, G. H., *J. Exptl. Med. 89,* 359 (1949).

70. ALLISON, J. B., *Advances in Protein Chemistry 5,* 155 (1949).

71. SWANSON, P. P., *Ann. Rev. Biochem. 19,* 235 (1950).

72. SCHWIMMER, P. and McGavack, T. H., *N. Y. J. Med. 48,* 1797 (1948).

73. SPECTOR, H., and Calloway, D. H., *Fed. Proc. 12,* 430 (1953).

74. BEATTIE, J., Herbert, P. H., and Bell, D. H. *Brit. J. Nutrition 1,* 202 (1947).

75. ROSENTHAL, H. L., and Allison, J. B., *J. Nutrition 44,* 423 (1951).

76. COX, WM., JR., Ellingson, R. C., and Mueller, G. J., Pediatrics, in press.

ABOUT THE AUTHOR

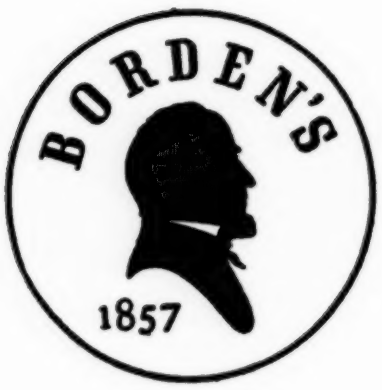
Although Prof. James Allison has spent most of his life in academic instit
either as a student or a teacher, he is far from the "ivory tower" model o
University Professor. He is a realist who understands and appreciates the impo
of developing and training students, not only for the academic arena, but fo
industrial and commercial research laboratories that have contributed so signifi
to our knowledge of chemistry and medicine. After a long, hard, and still conti
struggle, Jim Allison has in great measure met this challenge through the establish
of the Bureau of Biological Research at Rutgers University. This organization
veloped and directed under the guidance of Dr. Allison, does an excellent j
bringing the student, the teacher, the University and Industry together on one co
ground — the desire for training and the search for new knowledge. Those o
who have had an opportunity to visit the Bureau of Biological Research can be
preciate the friendly, helpful attitude that is so evident there — visitors are
to feel at home.

Dr. Allison received his undergraduate training at Pennsylvania State C
and his graduate training at Iowa State College which granted him the Ph.D. d
in 1927. He soon returned east, to Rutgers University, as an instructor in Biochem
Dr. Allison has stayed on at Rutgers and made significant contributions in the dev
ment of a well-rounded educational and research program in Biochemistry. He
made Professor of Biochemistry and Physiology in 1945, and also served as lectu
Physiology at the College of Physicians and Surgeons, Columbia University, 194

His long and continued interest and work in biochemistry and nutrition is ev
by his extensive list of publications and has earned him wide-spread recognition i
field of protein metabolism. Only recently he was invited to speak at a symposium
in Turkey on the disease Kwashiorkor.

We are happy to have Dr. Allison as one of our contributors.

— THE EDITOR



Printed in U.S.A.